

mechanisms. Some proteins and peptides are considered to be important in these mechanisms. The hypothesis for this newly started project, is that proteome changes during storage of carrots are related to the susceptibility to *M. acerina*. The carrots used in this study are grown under four different agricultural practices (one conventional and three organic), in order to investigate the effect of the cropping system on the susceptibility to liquorice rot. We are developing bioassays for infection studies of *M. acerina* on conventionally and organically cropped carrots in order to determine the critical time points in the infection process. The proteome of the carrots and of *M. acerina* will then be investigated at these different time points using two dimensional gel electrophoreses and mass spectrometry. Finally bioinformatics will be applied to shed light on the processes of infection and resistance during storage. We are developing bioassays for infection studies of *M. acerina* on conventionally and organically cropped carrots in order to determine the critical time points in the infection process. The proteome of the carrots and of *M. acerina* will then be investigated at these different time points using two dimensional gel electrophoreses and mass spectrometry. Finally bioinformatics will be applied to shed light on the processes of infection and resistance during storage.

#### PS 10-446

#### IDENTIFICATION OF VIRULENCE DETERMINANTS OF *PECTOBACTERIUM ATROSEPTICUM* BY PROTEOMICS

Laura MATTINEN<sup>1</sup>, Riitta NISSINEN<sup>1</sup>, Tero RIIPPI<sup>2</sup>, Nisse KALKKINEN<sup>2</sup> and Minna PIRHONEN<sup>1</sup>

<sup>1</sup>Department of Applied Biology, Plant Pathology Laboratory, University of Helsinki, Finland; <sup>2</sup>Institute of biotechnology, Protein Chemistry Laboratory, Helsinki, Finland.  
laura.mattinen@helsinki.fi

*Pectobacterium atrosepticum* (*Pa*) is a gram-negative bacterium that causes blackleg and soft rot of potato. It causes major economical losses in agriculture and potato processing industry in cool and temperate countries worldwide. In this study proteomics was used to characterize the secreted proteins, the secretome, of *Pa* strain SCRI1043. The bacteria were grown in minimal nutrient medium in low temperature to mimic the conditions where *Pa* normally causes symptoms in potato. Potato tuber extract or stem extract was added to induce the production of host specific proteins. Secretion pathways were studied using mutant strains. Our results suggest that the used growth conditions, low temperature and minimal medium supplemented with plant extract, may induce a unique combination of proteins in *Pa*. We identified 40 proteins, among them many known virulence factors but also several proteins with unknown function. Comparison of secretomes produced with or without potato extract revealed a group of proteins which seemed to be host induced. These conserved proteins were homologous with the so called Hemolysin coregulated proteins (*Hcp*). *Hcps* are reported to be involved in virulence in some animal pathogenic bacteria but their exact molecular function is yet unknown. *Pa* genome contains seven *Hcp* genes and four of them appeared in our analysis. To study if *Hcps* are virulence related we constructed a *Hcp*-overproducing mutant. The mutant was found to be over two times more efficient in macerating potato tubers than wild type. Our future goal is to determine the role of *Hcps* in virulence and study their function, structure and secretion pathway.

#### PS 10-447

#### UTILIZING THE COMPOSITAE GENOME PROJECT TO DISSECT THE GENOMIC ARCHITECTURE OF DISEASE RESISTANCE IN LETTUCE

Leah MCHALE, María José TRUCO, Alexander KOZIK, Tadeusz WROBLEWSKI, Dean LAVELLE, Oswaldo OCHOA, Kirsten LAHRE, Smitha MATHRAKOTT and Richard MICHELMORE

The Genome Center, University of California, Davis CA 95616, USA  
rwmichelm@ucdavis.edu

The Compositae Genome Project (CGP) has expanded the number of ESTs sequenced within the Compositae to over 700K ESTs. These sequences come from eighteen species including cultivated and wild types from the *Lactuca*, *Helianthus*, *Cichorium*, *Carthamus*, *Centaurea* and *Taraxacum* genera (<http://cgpdb.ucdavis.edu/>). Over 40,000 unigenes have been generated for cultivated lettuce, *L. sativa* and its wild progenitor, *L. serriola*. Disease resistance is being studied in detail, among other traits important in domestication and weediness. 720 candidate genes have been identified by mining the EST database for sequences with similarity to genes involved in disease resistance in other species and through PCR with degenerate oligonucleotide primers. These candidate genes were categorized as resistance gene candidates (RGCs), cell wall penetration genes, host factors, resistance signaling pathway genes, and defense response genes. Approximately 270 candidate genes have been mapped relative to 33 phenotypes for resistance to diverse pathogens, providing a global view of the architecture of disease resistance in lettuce for efficient breeding and functional studies. Candidate genes are distributed across the nine chromosomes of lettuce. RGCs are more often associated with phenotypes than other types of sequences and map into clusters. Taking advantage of tandem duplications of NBS-LRR type RGCs which co-localize with clusters of phenotypic loci, RNAi transgenics are being used to silence families of closely-related genes. These transgenics are being used as genetic tester stocks to determine which phenotypes in a cluster are encoded by similar sequences.

#### PS 10-448

#### LARGE SCALE EXPRESSION ANALYSIS OF SEQUENCES FROM *THEOBROMA CACAO-MONILIOPHTHORA PERNICIOSA* INTERACTION.

Braz Tavares da HORA JÚNIOR<sup>1</sup>, Abelmon da SILVA GESTEIRA<sup>1</sup>, Karina PERES GRAMACHO<sup>2</sup>, Júlio César de MATOS CASCARDO<sup>1</sup>, Sonia Marli ZINGARETTI DI MAURO<sup>3</sup>, Fabienne MICHELI<sup>1,4</sup>

<sup>1</sup>Laboratório de Genômica e Expressão Gênica, UESC, Ilhéus-BA, Brazil; <sup>2</sup>Laboratório de Fitopatologia Molecular, CEPEC, Ilhéus-BA, Brazil; <sup>3</sup>Brazilian Clone Collection Center, Departamento de Tecnologia, FCAV-UNESP, Jaboticabal-SP, Brazil; <sup>4</sup>Cirad-BIOS, UMR DAP, Montpellier, France.  
fabienne.micheli@cirad.fr, fabienne@uesc.br

The hemibiotrophic basidiomycete *Moniliophthora perniciosa* (Stahel) Aime & Phillips-Mora is the causal agent of the witches' broom, the main disease affecting the cacao production (*Theobroma cacao* L.) in Brazil. The decrease of cacao production in this country was responsible for important social, economical and ecological problems. Studies of functional genomics have been developed in our lab to better understand the cacao-*M. perniciosa* interaction and to develop effective methods for genetic breeding. To identify genes involved in *T. cacao* resistance



and in biological events associated to fungal virulence, we developed macroarrays from resistant and susceptible interaction cDNA libraries (2500 cDNA clones). The probes were obtained from resistant and susceptible cacao plants inoculated or not with *M. perniciosa* during the disease time course, adding an *in vitro* transcription step in their synthesis. Up and down regulated genes between genotypes, plant treatments and time course were identified. A general scheme of signalization pathways of defence is under construction and expression of some members of each cluster is under confirmation by qRT-PCR. We report here the first large scale expression analysis of *T. cacao-M. perniciosa* interaction, contributing to understand the resistance and susceptible mechanisms of this complex interaction.

#### PS 10-449

#### IN SILICO MULTI-SPECIES COMPARISONS OF PUBLICLY AVAILABLE EST DATA TO IDENTIFY GENES PUTATIVELY INVOLVED IN RESPONSE OF TOMATO TO STRESS.

Laura MIOZZI<sup>1</sup>, Paolo PROVERO<sup>2</sup>, Emanuela NORIS<sup>1</sup> and Gian Paolo ACCOTTO<sup>1</sup>

<sup>1</sup>Institute of Plant Virology, CNR, 10135 Turin, Italy; <sup>2</sup>Molecular Biotechnology Center and Dept. of Genetics, Biology and Biotechnology, University of Turin, 10126 Torino, Italy.

lmiozzi@ivv.cnr.it

Large-scale sequencing and analysis of Expressed Sequence Tags (ESTs) remains a fundamental part of genomics and post-genomics research to enable gene discovery and annotation. EST data are particularly important for organisms whose genomes are not yet sequenced and for which other expression profiling techniques have not yet been extensively applied. At the moment, millions of ESTs, collectively representing a variety of biochemical and functional states, are available in public databases such as dbEST-NCBI (<http://www.ncbi.nlm.nih.gov/dbEST/>) and TGI (<http://compbio.dfci.harvard.edu/tgi/>). This impressive wealth of information constitutes a valuable resource for comparative transcriptomic analysis, but has so far been exploited only in part.

Here we present an EST data mining strategy based on comparative meta-analysis. The method was applied to the Solanaceae family in an attempt to identify genes involved in the response of tomato to stress. This is possible due to the large number of ESTs available for at least some members of the family. The method consists in ranking tomato ESTs co-expressed with a gene of interest according to the level of expression pattern conservation in related plants (potato, pepper and tobacco), to obtain a list of genes putatively functionally related to that gene. The candidate genes are then analyzed for Gene Ontology keyword overrepresentation and related to available information from the literature. Here we present an EST data mining strategy based on comparative meta-analysis. The method was applied to the Solanaceae family in an attempt to identify genes involved in the response of tomato to stress. This is possible due to the large number of ESTs available for at least some members of the family. The method consists in ranking tomato ESTs co-expressed with a gene of interest according to the level of expression pattern conservation in related plants (potato, pepper and tobacco), to obtain a list of genes putatively functionally related to that gene. The candidate genes are then analyzed for Gene Ontology keyword overrepresentation and related to available information from the literature.

#### PS 10-450

#### HOST-INDUCED GENE SILENCING IN *BLUMERIA GRAMINIS*

Daniela NOWARA, and Patrick SCHWEIZER

Institute of Plant Genetic and Crop Plant Research (IPK), D-06466 Gatersleben, Germany

nowara@ipk-gatersleben.de

The powdery mildew-fungus *Blumeria graminis* f. sp. *hordei* (*Bgh*) potentially causes considerable losses in barley cultivation. Besides its agronomic importance, the barley-*Bgh* interaction may serve as model for many powdery mildew diseases on thousands of different hosts. Therefore, intensive research is going on to better understand this powdery mildew interaction. By using a recently established RNAi-based high-throughput phenomics screen for differentially-regulated barley genes affecting basal resistance, we observed resistance of host cells that contained constructs targeting mRNAs of *Bgh*. These pathogen-encoded sequences were derived from a *Bgh*-barley interaction library (ID-"HO") and selected due to differential hybridization signals on a cDNA array. In order to test the general applicability of the principle of "Host-Induced Gene Silencing" (HIGS) we targeted about 60 *Blumeria*-encoded genes by RNAi-constructs and used them for single-cell RNAi in barley epidermis. Eight of these constructs significantly reduced the colonization of transformed cells by *Bgh*. HIGS was also tested in the *Triticum aestivum*-*Blumeria graminis* f. sp. *tritici* (*Bgt*) system by introducing RNAi constructs against the *Bgt*-encoded orthologous genes of our eight *Bgh* candidates into wheat leaves. Two candidates also reduced the colonization of wheat epidermal cells by *Bgt* significantly. We were able to reproduce the HIGS-effect with Virus-Induced Gene Silencing (VIGS) and are currently testing transgenic barley plants expressing RNAi constructs directed against two of the identified *Bgh* genes.

Although the mechanism of HIGS is currently unclear, the phenomenon allows for functional studies by loss-of-gene function in an obligate biotrophic organism, and might offer a novel method for plant protection.

#### PS 10-451

#### THE *MALUS* - *VENTURIA INAEQUALIS* INTERACTION: RECENT ADVANCES IN THE COMPREHENSION OF THE MOLECULAR BASIS OF APPLE SCAB RESISTANCE.

Roberta PARIS<sup>1</sup>, Valentina COVA<sup>2</sup>, Giulia PAGLIARANI<sup>1</sup>, Fabrizio CARBONE<sup>3</sup>, Stefano TARTARINI<sup>1</sup>, Matteo KOMJANC<sup>2</sup>, and Silvano SANSVINI<sup>1</sup>

<sup>1</sup>Department of Fruit Tree and Woody Plant Sciences, University of Bologna, 40127 Bologna, Italy; <sup>2</sup>Istituto Agrario di S. Michele all'Adige, 38010 San Michele all'Adige (Trento), Italy; <sup>3</sup>R.C. Trisaia ENEA, Rotondella (Matera), Italy.

rparis@agrsci.unibo.it

The interaction of *Malus* genotypes and *Venturia inaequalis* (Cke.) Wint., the causal agent of apple scab, is one of the most studied plant-pathogen interaction involving a fruit tree species. Apple scab is the most damaging fungal disease affecting commercial apple production and scab resistance is nowadays one of the primary goal in all apple-breeding programs.

An apple scab resistance gene, named *HcrVf2* (homologue of the *Cladosporium fulvum* resistance genes of the *Vf* region), was recently cloned from the resistant cv. Florina. The *HcrVf2* gene encodes for a putative LRR receptor-like protein that is